

**IDENTIFICATION OF *ESCHERICHIA COLI* GENES REQUIRED FOR
BACTERIAL SURVIVAL AND MORPHOLOGICAL PLASTICITY IN
URINARY TRACT INFECTIONS**

DANIEL GIUSEPPE MEDIATI

A thesis submitted in fulfilment of the
requirements for the degree of
Doctor of Philosophy



The ithree institute and School of Life Sciences,
University of Technology Sydney

November 2018

[blank page]

CERTIFICATE OF AUTHORSHIP AND ORIGINALITY

I, Daniel G. Mediati, certify that the work in this PhD thesis, carried out between March 2014 to July 2018, has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree except as fully acknowledge within the text.

I also certify that this PhD thesis has been written by me. Any assistance that I have received in my research work and preparation of this thesis has been acknowledged. In addition, I certify that all information sources and literature used are indicated in this thesis.

This PhD was supported by an Australian Government Research Program Scholarship, formerly known as the Australian Postgraduate Award Scholarship until 2017.

Signature of candidate:

Production Note:

Signature removed prior to publication.

Daniel Giuseppe Mediati

Date: 30/11/2018

TO MUM AND DAD,
WHO INSTILLED IN ME THE GREAT VALUE OF
EDUCATION.

CONTENTS IN BRIEF

CHAPTER 1: INTRODUCTION

CHAPTER 2: GENERAL MATERIALS AND METHODS

CHAPTER 3: HIGH-THROUGHPUT SEQUENCING OF SORTED EXPRESSION LIBRARIES REVEALS INHIBITORS OF BACTERIAL CELL DIVISION

CHAPTER 4: GENOME-WIDE COMPARISON OF *E. COLI* GENES REQUIRED FOR GROWTH IN COMPLEX AND MINIMAL MEDIA

CHAPTER 5: THE ESSENTIAL GENE PROFILE SET OF UROPATHOGENIC *E. COLI* DURING BLADDER CELL INFECTION

CHAPTER 6: DISCUSSION AND CONCLUSION

REFERENCES

TABLE OF CONTENTS

Certificate of authorship and originality	iii
List of figures and supplementary data	x
List of tables and supplementary data	xi
Abbreviations	xii
Publications	xiv
Conference proceedings	xv
Acknowledgements	xvi
Preface	xvii
 CHAPTER 1: INTRODUCTION	 1
1.1 Urinary tract infections	3
<i>1.1.1 Uropathogenic Escherichia coli</i>	4
1.2 UPEC lifestyle from the intestine to the urinary tract	6
1.3 Infection cycle events in the bladder	8
<i>1.3.1 Bacterial attachment and invasion of host cells</i>	8
<i>1.3.2 Intracellular bacterial communities</i>	13
<i>1.3.3 UPEC dispersal and filamentation</i>	16
1.3.3.1 <i>E. coli</i> cell division and filamentation	19
1.3.3.2 Negative regulatory systems of cell division in <i>E. coli</i>	22
1.3.3.3 The SOS response and involvement of Sula	23
1.3.3.4 DamX and reversible filamentation during UTIs	26
1.4 UPEC metabolic responses during pathogenesis	27
<i>1.4.1 The primary medium: nutritional aspects of urine</i>	27
<i>1.4.2 Central carbon metabolism</i>	28
<i>1.4.3 Amino acid catabolism</i>	31
<i>1.4.4 Iron uptake and transport systems</i>	31
<i>1.4.5 Other metabolic regulators of UPEC</i>	33
1.5 Host immune responses to UPEC infection	34
<i>1.5.1 UPEC evasion of host defences</i>	34
1.6 ‘Omic screening approaches to further understand UTIs	35
<i>1.6.1 Comparative bioinformatics</i>	35
<i>1.6.2 Transcriptomics</i>	37
<i>1.6.3 Functional genomics</i>	38
1.7 Aims and objectives	40
 CHAPTER 2: GENERAL MATERIALS AND METHODS	 42
2.1 Bacterial strains and plasmids	43

2.2 Bacterial and mammalian growth media	44
2.3 Chemicals, reagents and solutions	45
2.4 General growth conditions of <i>E. coli</i>	46
2.4.1 <i>Preparation and transformation of E. coli</i>	46
2.5 General mammalian growth conditions	47
2.5.1 <i>General growth conditions of PD07i bladder cell infection model</i>	47
2.6 General methods and protocols used in this thesis	48
2.6.1 <i>Preparation and extraction of DNA from bacteria</i>	48
2.6.2 <i>Polymerase chain reaction (PCR)</i>	48
2.6.3 <i>Agarose gel electrophoresis</i>	49
2.6.4 <i>General multiplex DNA sequencing preparation</i>	49
2.6.5 <i>General analysis of DNA sequencing data</i>	50
 CHAPTER 3: HIGH-THROUGHPUT SEQUENCING OF SORTED EXPRESSION LIBRARIES REVEALS INHIBITORS OF BACTERIAL CELL DIVISION	 51
3.1 Introduction	53
3.2 Results	55
3.2.1 <i>A high-throughput screen based on flow cytometric sorting of DNA expression libraries</i>	55
3.2.2 <i>Identifying genomic regions that encode mediators of reversible bacterial filamentation</i>	60
3.2.3 <i>Identification and verification of DNA fragments that cause filamentation</i>	67
3.3 Discussion	73
3.4 Methods	79
3.4.1 <i>Bacterial strains and plasmids</i>	79
3.4.2 <i>Construction of the shotgun UTI89 genomic DNA expression library</i>	79
3.4.3 <i>Cell sorting for enrichment of filamentous clones from the expression library</i>	80
3.4.4 <i>High-throughput multiplex DNA sequencing and data analysis</i>	80
3.4.5 <i>Expression of cloned ORFs and analysis of cell phenotypes</i>	82
3.4.6 <i>Amplification and sub-cloning of fragments from identified genomic regions</i>	82
 CHAPTER 4: GENOME-WIDE COMPARISON OF <i>E. COLI</i> GENES REQUIRED FOR GROWTH IN COMPLEX AND MINIMAL MEDIA	 84
4.1 Introduction	86
4.2 Results	88
4.2.1 <i>Design of a modified TraDIS protocol</i>	88
4.2.2 <i>Construction of a transposon mutant library in UTI89</i>	89

4.2.3 <i>Genes advantageous and disadvantageous for growth</i>	92
4.2.4 <i>Identification of genes required for growth in M9-glycerol minimal medium</i>	95
4.2.5 <i>Verification of genes required for growth in M9</i>	102
4.2.6 <i>Comparison of a subset of genes to E. coli K-12</i>	104
4.3 Discussion	108
4.4 Methods	114
4.4.1 <i>Bacterial strains and growth conditions</i>	114
4.4.2 <i>Construction of a mini-Tn5 transposon mutant library in UTI89</i>	114
4.4.3 <i>Mutant selection in minimal media</i>	115
4.4.4 <i>Transposon-directed insertion-site sequencing (TraDIS)</i>	116
4.4.5 <i>Analysis of nucleotide sequence data</i>	116
4.4.6 <i>Statistical analyses for identifying essential genes</i>	117
4.4.7 <i>Verification of individual deletion mutants</i>	117
4.4.8 <i>Transposon mutant library passage</i>	118
4.4.9 <i>Human bladder cell culture model of UTI</i>	119
CHAPTER 5: THE ESSENTIAL GENE PROFILE SET OF UROPATHOGENIC E. COLI DURING BLADDER CELL INFECTION	120
5.1 Introduction	121
5.2 Results	123
5.2.1 <i>Development of an up-scaled infection model to assay UPEC gene essentiality during UTIs</i>	123
5.2.2 <i>The essential gene profile set of UPEC during in vitro infection</i>	130
5.2.2.1 The genes required for progression to IBCs	135
5.2.2.2 The genes required for UPEC dispersal and filamentation	141
5.2.2.3 The genes required for filament reversal and bacterial recovery from dispersal	151
5.3 Discussion	159
5.4 Methods	164
5.4.1 <i>Bacterial strains and growth conditions</i>	164
5.4.2 <i>Development of an up-scaled in vitro UTI model</i>	164
5.4.2.1 IBC phase of infection	164
5.4.2.2 Dispersal and filamentation phase of infection	165
5.4.3 <i>Mutant harvest from infection for TraDIS analysis</i>	166
5.4.3.1 Pre-infection (inoculate) sample	166
5.4.3.2 IBC sample	167
5.4.3.3 Dispersed bacterial sample	167
5.4.3.4 Non-dispersed bacterial sample	167
5.4.3.5 Recovery samples	168

5.4.4 Transposon-directed insertion-site sequencing (<i>TraDIS</i>)	168
5.4.5 Analysis of nucleotide sequencing data	168
CHAPTER 6: DISCUSSION AND CONCLUSION	170
6.1 General Discussion	171
6.1.1 Reversible filamentation in <i>UPEC</i>	173
6.1.2 Metabolic adaptations and requirements of <i>UPEC</i>	175
6.1.3 Future directions	176
6.1.4 Concluding remarks	177
APPENDIX: SUPPLEMENTARY DATA	179
REFERENCES	188

LIST OF FIGURES AND SUPPLEMENTARY DATA

- Figure 1.1** Well characterised virulence factors of uropathogenic *E. coli*.
- Figure 1.2** UPEC pathogenesis from the intestine to the urinary tract and bloodstream.
- Figure 1.3** Type-1 pilus and the adhesin protein FimH-mediated binding.
- Figure 1.4** Mouse bladder epithelial cell endocytosis of UPEC.
- Figure 1.5** Intracellular bacterial communities of UPEC within the mouse bladder.
- Figure 1.6** Bacterial filamentation, egress and dispersal from the bladder cell.
- Figure 1.7** Simplified overview of *E. coli* cell division and the main known negative regulators of Z-ring formation.
- Figure 1.8** FtsZ assembly during cell division and the involvement of SulA.
- Figure 1.9** Some important metabolic pathways of UPEC during urinary tract infection.
- Figure 3.1** Analysis and purification of filamentous bacteria from the UTI89 DNA-expression library by flow cytometry.
- Figure 3.2** High-throughput DNA sequencing of plasmid libraries from the reference-unsorted and filamentous-sorted populations.
- Figure 3.3** Overexpression of the *pptE* and *pdhR* ORFs cause *E. coli* filamentation independent of *recA* (SOS response).
- Figure 3.4** Partial fragments of ORFs cause filamentation.
- Figure 4.1** Genomic overview of the UTI89 transposon mutant library.
- Figure 4.2** Mutant compositional changes during library growth in LB.
- Figure 4.3** Number of essential genes according to COG functional category.
- Figure 4.4** Identification of genes required for growth of UPEC in M9 by TraDIS.
- Figure 4.5** Verification and characterisation of deletion mutants in UTI89.
- Figure 4.6** Characterisation of deletion mutants in *E. coli* K12 identified by TraDIS.
- Figure 5.1** Up-scaled bladder cell infection model permits UTI89 IBCs.
- Figure 5.2** Characterisation of UPEC dispersal *in vitro* and filamentous cells.
- Figure 5.3** Schematic diagram of TraDIS approach.
- Figure 5.4** The numbers of genes identified as important to stages of infection by TraDIS, indicating the numbers of genes common to multiple stages.
- Figure 5.5** Identification by TraDIS of UPEC genes required for bladder infection.
- Supp. Data Figure S1** Additional analyses of DNA sequencing data.
- Supp. Data Figure S2** *pptE* overexpression causes major effects on cell structure.
- Supp. Data Figure S3** ORFs that do not cause substantial filamentation when overexpressed.
- Supp. Data Figure S4** Outline of the modified TraDIS DNA sequencing protocol.
- Supp. Data Figure S5** Correlation between the Tni5 and Tni7 DNA sequencing ends.
- Supp. Data Figure S6** Analysis of filamentous bacteria from the dispersal phase of infection.

LIST OF TABLES AND SUPPLEMENTARY DATA

Table 2.1 General *E. coli* strains used in this thesis.

Table 2.2 General plasmids used in this thesis.

Table 2.3 Bacterial and mammalian growth media.

Table 2.4 General chemical, reagents and solutions used in this thesis.

Table 2.5 Aqueous buffers and solutions used in this thesis.

Table 3.1 Read frequency from sequencing of plasmid libraries.

Table 3.2 UTI89 chromosomal regions causing filamentation – high stringency hits in both screens.

Table 3.3 Genomic FatI fragments from the *ybiY-moeA* and *ybeM-lipA* regions of UTI89 that caused a filamentous phenotype.

Table 4.1 UTI89 genes required for growth in M9-glycerol – high stringent hits

Table 5.1 UTI89 mutants that have a reduced ability to survive to IBC.

Table 5.2 UTI89 mutants that have a reduced ability to survive to the dispersal phase.

Table 5.3 UTI89 mutants that have a reduced ability to recover from dispersal.

The following Supplementary Data Tables can be accessed online with this thesis.

Supp. Data Table S1 Supplementary Data for the work in Chapter 3.

Supp. Data Table S2 Supplementary Data for the work in Chapter 4.

Supp. Data Table S3 Supplementary Data for the work in Chapter 5.

ABBREVIATIONS

A	Absorbance
Amp	Ampicillin
BLAST	Basic local alignment search tool
bp	Basepair
Cm	Chloramphenicol
Δ	Delta (change in)
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DTI	Defined trypsin inhibitor
g	Centrifugal force (x unit gravitational field)
GFP	Green fluorescent protein
h	Hours
IBC	Intracellular bacterial community
kb	Kilobase
Km	Kanamycin
L	Liters
LB	Luria Broth
M	Molarity (mol/L)
m	Meters
μ	micro (10^{-6})
Min	The Min protein system
min	Minutes
mL	Mililiters
MOI	Multiplicity of infection
ms	Milliseconds
MW	Molecular weight
n	nano (10^{-9})
NO	Nucleoid occlusion system
OD	Optical density
ORF	Open reading frame
ρ	Density (Rho)
PAI	Pathogenicity island
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
QIR	Quiescent intracellular reservoir
RNA	Ribonucleic acid
RT	Room temperature
rpm	Revolutions per minute

TBE	Tris/borate/EDTA
TraDIS	Transposon-directed insertion-site sequencing
UPEC	Uropathogenic <i>Escherichia coli</i>
UTI	Urinary tract infections
V	Volts
v	Volume
w	Weight
WT	Wild-type

PUBLICATIONS

This doctoral thesis is a compilation of the work carried out as part of the project “Identification of *Escherichia coli* genes required for bacterial survival and morphological plasticity in urinary tract infections” which resulted in the following written works:

Mediati, D.G., Iosifidis, G. (2016) Lifestyle dynamics of uropathogenic *Escherichia coli* in the urinary tract. *ASM Syntrophy*, 17:3.

Mann, R., Mediati, D.G., Duggin, I.G., Harry, E.J., & Bottomley, A.L. (2017) Metabolic adaptations of uropathogenic *E. coli* in the urinary tract. *Frontiers in Cellular and Infection Microbiology*, 7:241.

Mediati, D.G., Burke, C.M., Ansari, S., Harry, E.J., Duggin, I.G. (2018) High-throughput sequencing of sorted expression libraries reveals inhibitors of bacterial cell division. *BMC Genomics*, 19:781.

Mediati, D.G., Monahan, L.G., Charles, I.G., Duggin, I.G. (2018) Genome-wide comparison of *E. coli* genes required for growth in complex and minimal media. *Journal of Bacteriology*, (manuscript to be submitted).

CONFERENCE PROCEEDINGS

This doctoral thesis is a compilation of the work carried out as part of the project “Identification of *Escherichia coli* genes required for bacterial survival and morphological plasticity in urinary tract infections” which resulted in the following presentations:

East Coast Bacillus Meeting, Wollongong, NSW, Australia 2014

Oral Presentation

Authors: Mediati, D.G., Iosifidis, G., Duggin, I.G.

40th Annual Infection and Immunity, Lorne, VIC, Australia 2015

Poster Presentation

Authors: Mediati, D.G., Duggin, I.G.

13th Annual Bacterial Pathogenesis, Phillip Island, VIC, Australia 2015

Oral Presentation

Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, L., Duggin, I.G.

University of Technology Sydney: 3-minute thesis competition 2015

Oral Presentation

Authors: Mediati, D.G., Duggin, I.G.

East Coast Bacillus Meeting, Wollongong, NSW, Australia 2015

Oral Presentation

Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, L., Duggin, I.G.

Becton-Dickinson (BD) Award Final, Sydney, NSW, Australia 2016

Oral Presentation

Authors: Mediati, D.G., Burke, C., Ansari, S., Harry, E., Duggin, I.G.

East Coast Bacillus Meeting, Sydney, NSW, Australia 2016

Oral Presentation

Authors: Mediati, D.G., Monahan, L.G., Charles, I.G., Duggin, I.G.

14th Annual Bacterial Pathogenesis, Adelaide Hills, SA, Australia 2017

Poster Presentation

Authors: Mediati, D.G., Monahan, L.G., Charles, I.G., Duggin, I.G.

ACKNOWLEDGEMENTS

First and foremost acknowledgement goes to Associate Professor Iain Duggin, for supervision of the work contributing to this PhD thesis. I cannot thank you enough for believing in me and giving me the opportunity to join your research team. You have been an excellent mentor and friend to learn from. Thank you for all your advice and support over the years.

I am also grateful for help from the following people: Leigh Monahan, for all your advice on the TraDIS technique and discussions on *E. coli* metabolism; Michael Liu, for all your assistance and guidance on sequencing and DNA library preparations; Greg Iosifidis, who taught me everything about mammalian tissue culturing and microscopy; Catherine Burke, who played a central role in the flow cytometry sorting and analysis results within Chapter 3; Elizabeth Harry, for all your insights into antimicrobial resistance and, together with the members of the Harry lab, who always provided great discussion and insight into bacterial cell division.

Finally, thank you to my parents and family. Mum and Dad, you have always encouraged and supported all of my academic endeavors. Without your guidance and love, I would have never had the courage to overcome the adversities I have faced in life. This PhD would not have been accomplished without the both of you.

PREFACE

Urinary tract infections (UTIs) are the second most common bacterial infectious disease affecting humans, after pneumonia. A range of pathogens have been implicated in causing UTIs, however strains of uropathogenic *Escherichia coli* (UPEC) are the predominant etiological agents. UPEC originate within the intestine, but have adapted the ability to disseminate and colonise the human urinary tract via a multi-stage intracellular infection cycle within the cells of the bladder. This infection cycle is a complex pathway involving epithelial cell attachment, invasion and intracellular biofilm-like proliferation, leading to the formation of a sub-population of filamentous bacteria. Bacterial filamentation occurs when rod-shaped cells grow without dividing. This filamentation accompanies bacterial dispersal and the rupture of the host bladder cell. UPEC filaments have the potential to revert to bacillary, rod-shaped morphology and can thereafter divide as normal bacterial cells, thereby initiating a new infection cycle. There is also the potential for UPEC to ascend to the kidneys and enter the bloodstream causing urosepsis.

The rapid emergence of antibiotic resistance has greatly influenced the severity of UTIs and financial burden on the health-care sector. This further complicates UTI therapies and highlights the urgent need to advance our understanding of the biological mechanisms and requirements underpinning UPEC survival and pathogenesis.

The work carried out for this PhD thesis aimed to expand our existing knowledge surrounding UPEC survival and morphological plasticity (cell shape changes). Each Chapter within this thesis focused on distinct, but related aspects of this overarching aim. Firstly, we developed and applied a high-throughput sequencing-based method for the genome-wide identification of genes and genomic DNA fragments that induce filamentation in *E. coli*. This revealed genes from several prophages, carbon metabolic pathways, as well as endogenous bacterial genes or loci that have known and novel roles in cell division or bacterial filamentation. A large number of short predicted peptides that trigger filamentation were also identified for the first time, which are not expected to have evolved with the purpose of causing filamentation, but could be used as synthetic, artificial inhibitors of cell division.

A transposon-insertion mutant library in the uropathogenic *E. coli* cystitis isolate UTI89 was constructed at a moderate scale. We combined this library with a modified transposon-directed insertion-site sequencing (TraDIS) technique to define the genes required for UPEC growth and survival in M9-glycerol minimal medium compared to a rich LB medium. We identified 60 mutants with a significant fitness defect and reduced capacity to survive in the M9-glycerol, the majority of which encode gluconeogenic and amino acid catabolism proteins. We also highlight novel differences and several apparent discrepancies in the metabolic requirements between uropathogenic and commensal *E. coli*. Several uncharacterised and UPEC-specific genes were identified that likely underlie metabolic capacities of UPEC strains during infection. Two of these genes, *neuC* and *hisF* were confirmed and verified as important during UPEC infection of human bladder epithelial cells *in vitro*.

Finally, we employed TraDIS to an up-scaled *in vitro* UTI model to identify the UTI89 genes required for survival at distinct phases of the multi-stage bladder infection cycle, focusing particularly on the later infection events, when bacterial physiology appears to most substantially change. This revealed a total of 143, 333 and 322 statistically significant genes required for the IBC, dispersal and recovery phases of bladder cell infection. We additionally characterised the extent and distribution of UPEC filamentation in the dispersal phase of our up-scaled model and implicate, through TraDIS, known and novel cell division regulators to potentially be involved in the UTI-filamentation response pathway. Further, the catalog of genes identified through our TraDIS experiments provides a foundation for further characterisation of UPEC factors needed for survival, both in laboratory culture conditions and during human bladder infection.

The extensive gene functional identification reported in this PhD thesis represents both a substantial resource and some of the first steps towards the understanding of UPEC gene function during infection. This is expected to facilitate the development of treatments for UTIs or other bacterial infections in a future faced with increasing resistance to current antibiotics.